Microscope scanning into the gel on the z-axis confirming the presence of a 3D cellular network within the hydrogel.

Pancreatic Cancer Cells Encapsulated in the Biologically Inert Hydrogel

[0215] Pancreatic ductal adenocarcinoma cells ($Kras^{G12D}$ and $p53^{R172H}$) were dispersed in the polymer bio-ink with no PEG-RGD at 2×10^6 cells/mL to yield the cell polymer bio-ink and the cell polymer bio-ink printed together with the activator with no MMP-2 responsive peptide. In situ encapsulation was achieved by directly 3D bioprinting the cell-bio-ink with the PEG-bis-thiol activator on the 96-well plate.

[0216] Confocal images showed pancreatic cancer cells encapsulated inside the hydrogel. Morphology of the cells was confirmed over the incubation period. Due to the lack of RGD and MMP-2 cross-linker, cells were rounded in morphology inside the gel. This morphology indicated that the cells were unable to interact with the hydrogel to generate focal adhesion, and were unable to move inside the gel. Live/dead assay was conducted on the encapsulated cells after 3 days incubation period. Fluorescence images showed viable cells (>95% viability) after 3 days of incubation.

Pancreatic Cancer Cells Encapsulated in the Biologically Active Hydrogel

[0217] Pancreatic ductal adenocarcinoma cells ($Kras^{G12D}$ and $p53^{R172H}$) were dispersed in the cell polymer bio-ink at 2×10^6 cells/mL and printed together with the MMP-2 responsive peptide activator (at 50:50 thiol concentration ratio). In situ encapsulation was achieved by directly 3D bioprinting the cell polymer bio-ink with the PEG-bis-thiol and MMP-2 activator on the 96-well plate. The MMP-2 activator was used to allow cellular invasion.

[0218] Confocal images showed pancreatic cancer cells encapsulated inside the hydrogel. Morphological changes of the cells were confirmed over the incubation period. Inside the gel, cell-cell interactions were represented by the formation of cell clusters, while cell-gel interactions and cell invasion were confirmed by the presence of cellular membrane protrusion. Live/dead assay was conducted on the encapsulated cells after 3 and 6 days incubation period. Bright field and fluorescence mages showed viable cells (>98% viability) after 3 days of incubation and confirmed the presence of cell-cell and cell-gel interactions. Cellular invasion was more pronounced at 6 days incubation, indicating strong cell-gel interactions. At the same time, cellular viability of >98% was also maintained after 6 days incubation inside the gel.

MCF7 Breast Cancer Cells Seeded in the Hydrogel

[0219] Cellular activities were confirmed via observation of cell morphologies under a bright-field microscope after 3-day incubation. Aggregation of single MCF7 cells were visible after the incubation period, forming multiple spheroids in the pre-defined gel area, indicating the ability of individual MCF7 cells to move to each other inside the hydrogel.

Example 15—Modification of Gelatin from Cold Fish Skin with Maleimide Group

[0220] Gelatin from fish skin (1 g) was dissolved in 20 mL of 2-(N-morpholino)ethanesulfonic acid (MES) buffer at 40° C. In a separate container, 6-maleimidohexanoic acid (MHA; 0.211 g), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, at 1:20 EDC:MHA molar ratio) and N-hydroxysuccinimide (NHS, at 1:2 NHS:EDC molar ratio) were dissolved in 1 mL MES buffer and left to stir for 20 minutes to activate the carboxylic acid (COOH) groups. The solution of activated COOH group was then transferred into the gelatin solution and the reaction was left to proceed for 24 h. The resulting solution was purified via dialysis against 10 mM hydrochloric acid (HCl) and 1 wt % sodium chloride (NaCl) for 2 days, followed by dialysis against 10 mM HCl for 1 day. The purified gelatin solution was then freeze dried to yield maleimide modified fish gelatin.

Example 16—Modification of Gelatin from Porcine Skin with Maleimide Group

[0221] Gelatin from porcine skin (gel strength 300, type A, 1 g) was dissolved in 20 mL of MES buffer at 40° C. In a separate container, MHA (0.139 g), EDC (at 1:20 EDC: MHA molar ratio) and N-hydroxysuccinimide (NHS, at 1:2 NHS:EDC molar ratio) were dissolved in 1 mL MES buffer and left stirred for 20 minutes to activate the COOH groups. The solution of activated COOH group was then transferred into the gelatin solution and the reaction was left to proceed for 24 h. The resulting solution was purified via dialysis against 10 mM HCl and 1 wt % NaCl for 2 days, followed by dialysis against 10 mM HCl for 1 day. The purified gelatin solution was then freeze dried to yield maleimide modified porcine gelatin.

Example 17—Gelation of Maleimide Modified Gelatin

[0222] Maleimide modified gelatin from both porcine and cold fish skin, synthesised in Examples 15 and 16, were each dissolved in PBS at 20 wt % to yield the polymer bio-inks. The pH of each solution was neutralised to pH 7.4 with 1M NaOH. To prepare the activator, PEG-bis-thiol at equal SH molar concentration to the maleimide molar concentration was dissolved in PBS. Gelation was achieved by mixing equal volumes of each polymer bio-ink and activator.

Example 18—3D Bioprinting of Collagen-Containing PEG Hydrogel

Substrate

[0223] A cell culture dish supplied by Thermofisher was etched with a target grid mimicking the dimension of a 96-well plate.

Polymer Bio-Ink

[0224] To prepare polymer bio-ink, PEG-Mal (0.15 g) was dissolved in 1.5 mL PBS to yield a 10 wt % polymer bio-ink. The solution was filtered through a 0.22 μm syringe filter aseptically.

Activators

[0225] Collagen containing activator was prepared by mixing GCRDPLGLDRCG (0.01 g) in 0.5 mL PBS. Subsequently, 0.5 mL of Type I bovine collagen (3.1 mg) was mixed into the activator solution to yield a collagen containing MMP-activator. Subsequently, the pH of the solution